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14. ABSTRACT
Traumatic brain injury (TBI) is a serious public health issue for those in active military duty as well as the general public. TBI produces a host of short- and long-term consequences, including diffuse axonal injury, tau aggregation, increased amyloid burden, and reactive astrocytosis. Many of these pathologies overlap with those observed in Alzheimer's disease (AD), with a growing body of evidence suggesting that TBI is a risk factor for AD. Using a TBI induction protocol that effectively models the injury sustained from an explosive impact, a common source of TBI for military personnel, we seek to characterize the convergence of TBI and AD. We hypothesized that reactive astrocytosis underlie the shared pathway to neuronal pathologies seen in TBI and AD. To this end, our results show the expected behavioral outcome from the blasted mice, however it is still too early in the study to make any concrete conclusions. We will continue our efforts to describe the convergence of TBI and AD by looking into synaptic changes associated with blast in hippocampal neurons and alterations in morphology and physiology of hippocampal astrocytes.

15. SUBJECT TERMS
Traumatic brain injury, Alzheimer's disease, astrocytes, reactive astrocytosis, neuronal excitability, monocarboxylate transporters (MCT), lactate shuttling, excitatory amino acid transporters (EAAT), glutamate uptake

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INTRODUCTION:

Traumatic brain injury (TBI) is a serious public health issue for those in active military duty as well as the general public. TBI produces a host of short- and long-term consequences, including diffuse axonal injury, tau aggregation, increased amyloid burden, and reactive astrocytosis. Many of these pathologies overlap with those observed in Alzheimer's disease (AD), with a growing body of evidence suggesting that TBI is a risk factor for AD. Using a TBI induction protocol that effectively models the injury sustained from an explosive impact, a common source of TBI for military personnel, we seek to characterize the convergence of TBI and AD. Specifically, we hypothesize that reactive astrocytosis underlie the shared pathway to neuronal pathologies seen in TBI and AD. Although astrocyte reactivity serves to protect neurons, some normal astrocytic functions including lactate shuttling and glutamate uptake may be compromised during this process, leading to disruptions to neuronal health and proper synaptic function. If astrocytes play an active role in synaptic transmission, astrocytic impairments observed in TBI and AD will serve as the basis for future translational work.

KEYWORDS:

Traumatic brain injury, Alzheimer's disease, astrocytes, reactive astrocytosis, neuronal excitability, monocarboxylate transporters (MCT), lactate shuttling, excitatory amino acid transporters (EAAT), glutamate uptake

OVERALL PROJECT SUMMARY:

Aim 1 - To determine if TBI produces hallmarks of AD

Objective 1.1 To determine if TBI and AD converge on altered hippocampal function.

TBI induction and behavioral phenotyping

We have been performing blast-induced TBI on mice using a shock tube system since January 2014. Considerable effort has been made to determine the appropriate age range and pressure to use. A major challenge has been the genetic heterogeneity of the 5xFAD mice that were generated and maintained on a mixed C57BL/6J and SJL/J background. As hemizygous 5xFAD mice carrying the AD related allele with unknown genetic background were backcrossed with B6SJL wild type, this non-congenic line exhibits a range of phenotypes. For example, there is a large variability in their susceptibility to the TBI insult induced by blast. Accordingly, the mortality rate and behavioral performance as assessed with the classic fear conditioning paradigm were highly variable between experimental subjects.

To this end, we have performed blast and behavioral analysis of only the F1 (first filial generation) offspring of a cross between C57BL/6J females and SJL/J males—here abbreviate as B6SJLF1. This exercise confirmed that the variability we saw from the 5xFAD B6SJL mice is indeed a biological variance arising from the heterogeneous genomic content of each mouse rather than a technical issue. More importantly, we have gathered a complete dataset showing that blast consistently increases freezing behavior in B6SJLF1 mice. This is consistent with the post-traumatic stress disorder (PTSD) behavior commonly exhibited by soldiers and veterans with prior TBI insults. Moving forward, to maximize our ability to detect even small changes in our experiments, we are re-establishing our 5xFAD colony with a C57BL/6J congenic line. We expect to begin blasting these mice in the next month.

Diffusion tensor imaging (DTI)

We have begun our DTI analysis in recent months. A handful of mice were blasted and imaged longitudinally. The preliminary data are encouraging; we have observed qualitative differences in a wide area of the cortex. We are now in the process of doing the appropriate control experiments to ensure reliability of the measurements.

Objective 1.2 To determine if TBI and AD converge on altered histological profile.

Immunohistochemistry on sections from six-month wild-type and 5xFAD B6SJL mice were performed. Z-stacks were taken from the sensorimotor cortex, hippocampal CA1 and CA3 regions. As expected, we observed increased levels of Aβ plaques within the hippocampus in 5xFAD mice as compared to wild-type controls. Additionally, immunoreactivity for soluble Aβ oligomers and phosphorylated tau showed elevated levels in 5xFAD mice relative to the controls. We did not see consistent alterations in Aβ or phospho-tau levels in the blasted mice. As only moderate to severe TBI has a significant correlation with AD in humans, it is perhaps not surprising to see that closed-skull blast-induced TBI, which is a relatively mild form of TBI, has no overt pathology that resembles AD. Consistent with this, GFAP immunohistochemistry on sections from age-matched naïve and TBI mice did not reveal consistent astrocyte reactivity with our blast model. As most soldiers commonly receive

concomitant blast and percussion type injuries, it will be valuable to examine the mice that suffer from percussion type injuries and their potential additive effect with subsequent blast injuries.

Aim 2 - To determine if TBI and AD converge on altered transcriptomic profile in astrocytes

Objective 2.1 To determine if TBI and AD converge on altered transcriptomic profile in astrocytes.

We are experienced with using fluorescence activated cell sorting (FACS) to purify neurons from mice up to 9 month of age. However, we were not satisfied with the sorting of the astrocytes even after several attempts. While the physical properties of the cells appeared generally very similar to those in previously published work, we were not content with the purity of the approach, at least not without further optimization. The impurities in our samples were confirmed with gene expression analysis using quantitative PCR with cell type-specific markers. As there is a general trend in the scientific community of moving toward next generation sequencing for unbiased, whole-genome transcriptomic analysis, the suboptimal sample preparation will not likely yield useful information. For this reason, we have decreased our enthusiasm toward an extensive gene profiling effort at this time.

Objective 2.2 To determine if TBI and AD converge on altered astrocyte activity.

The major goal of this objective is to monitor astrocytic Ca^{2+} transients using GCaMP-based imaging approach. We have been successful in using this technology on other non-DoD funded projects. However, with the 5xFAD mice previously on the mixed B6SJL background, viral injection needs to be performed to deliver GCaMP. This is a rather low yield experiment, due to the uncertain mortality rate from the blast and the labor intensive injection procedures. Thus, we have placed our efforts on other fronts in order to maximize the readout from each experimental subject. That said, GCaMP3-imaging from astrocytes will be extremely feasible using a genetic approach in the future once the C57 congenic 5xFAD line is established.

Aim 3 - To determine if TBI and AD converge on altered neuronal support

Objective 3.1 To determine if TBI and AD converge on altered astrocyte-neuron metabolic coupling.

We hypothesized that TBI impairs metabolic support from astrocytes to neurons. This can be recapitulated when shuttling of energy substrate between these two cell classes is disrupted. To this end, adeno-associated viruses encoding short-hairpin RNA for monocarboxylate transporter 2 and 4 were generated. Quantitative PCR confirmed that these constructs induced downregulation of their corresponding target mRNA levels. Further experiments on this objective has been postponed as we did not observe a decreased neuronal activity following TBI induction. To summarize, the excitability of CA1 neurons from naive and blasted mice was carefully measured with whole-cell patch-clamp recordings. These cells did not show any alteration in the input-output function, i.e. equal number of spikes can be evoked in CA1 neurons from from both naive and blasted mice, with a given current injection. Accordingly, their maximal firing capacity is also similar between the two groups. Estimation of voltage-gated sodium channel availability confirm this inference. In addition, the excitability of CA1 neurons from wild type and 5xFAD B6SJL mice was also measured with whole-cell patch-clamp recordings. In contrast to what was seen in the blasted mice, these cells did show alterations in input-output function, with cells from 5xFAD mice showing a greater number of spikes for a given current injection and a significantly greater maximal firing capacity. The spikes in cells from 5xFAD mice are both shorter and narrower, suggesting an increased activity of repolarizing potassium conductances. Voltage clamp experiments will be performed in the future to pinpoint the potassium channel types involved.

Objective 3.2 To determine if TBI and AD converge on the level of altered synaptic regulation.

This is perhaps the most exciting front of our research. From our data it is clear that there is altered synaptic function in the blasted mice. Specifically, the relative contribution of AMPA and NMDA receptor content at the Schaffer collateral-CA1 (SC-CA1) synapse appeared to be different. The cause of this is not yet known. We currently hypothesize that the release of glycine and D-serine from astrocytes is impaired. As they are the co-agonists for NMDA receptors, this causes a deficit in NMDA receptor function at the SC-CA1 synapse. This idea is currently being pursued. As AMPA (but not NMDA) receptor density correlates tightly with spine volume, it is possible that spines that are small and have low AMPA content are preferentially lost following TBI. CA1 neurons have been dye-filled and imaged. We are currently examining if there is any spine loss in the blasted mice. In support of this hypothesis, occurrence of excitatory postsynaptic currents is decreased with blast. This is likely to be an underestimation, as the apparent release probability of the SC-CA1 synapse is increased with blast. Ultrastructural analysis with electron microscopy can be performed to carefully examine anatomical alterations associated with blast-induced TBI.

KEY RESEARCH ACCOMPLISHMENTS:

Nothing to report.

CONCLUSION:

As we have only begun to interrogate the effect of blast on cellular alterations in hippocampal astrocytes and neurons, it is still too early to make any concrete conclusions. Overall, we are seeing the expected behavioral outcome from the blasted mice. Specific experiments have been described throughout the report for readability reasons. The broad goals and directions of research in the next funding period are outlined below.

As we now have the TBI induction protocol established, we will continue to place our major focus on synaptic changes associated with blast. Specifically, we want to expand our analysis on the B6SJLF1 mice. Furthermore, alterations in astrocyte morphology and physiology will be performed. In addition to measurements of basic membrane properties, glutamate transporter function will be examined. Glutamate transporter function is of particular interest because of its immediate linkage to synaptic glutamate transmission. As outlined earlier, dendritic arborization pattern and spine density of CA1 neurons will monitored. The ramification of astrocytes will also be studied. As soon as the congenic C57 5xFAD mice come online, we will begin to blast and study the convergence of the etiology of TBI and AD using the established assays.

It will be important to compare and contrast the cellular alterations induced by blast against more severe TBI induction paradigm, e.g. closed-skull percussion type injuries. The additive/synergistic effect of both insults will be particularly relevant to soldiers and veterans.

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Nothing to report